CHAPTER 3
Inherent and flexible anti-predator behaviour and habitat choice in juvenile rainbowfish (*Melanotaenia spp.).*

3.1 ABSTRACT

Two complimentary experiments were conducted to examine if anti-predator responses in rainbowfish populations are inherent. In the first experiment, juvenile rainbowfish were bred from two stocks used in a previous study and their responses towards predators compared to examine the extent to which anti-predator responses are inherited. In the second experiment, juvenile rainbowfish from three locations were reared in the lab and transferred into structurally complex environments and bare tanks. The populations chosen for the experiments all differed from one another in terms of their anti-predator responses shown by adults. Predation pressure and the level of habitat complexity in their native environment may explain the observed differences in responses. The juveniles tested in the first experiment mirrored the responses displayed by their parents in an earlier study. While overall there was no significant effect of the rearing environment (complex structure or bare tank) on the use of the complex environment provided in the second experiment, there was an interaction between treatment and predator presence. Fish that had been reared in a bare tank showed a significant increase in their use of the complex habitat when exposed to a potential predator. Fish that had grown up in the complex environment spent a large amount of time in the complex habitat but did not alter their use when exposed to a predator. These data indicate that both predator avoidance responses and the use of habitat are primarily inherited, but may be slightly altered by individual experience.

3.2 INTRODUCTION

Brown and Warburton (1997) postulated that the anti-predator responses displayed by prey fish vary not only with the predator history of the populations and the experiences of individuals, but also with the degree of habitat complexity. Animals originating from predominantly open areas are likely to rely heavily on schooling to avoid predators, whereas animals from structurally complex areas are more likely to rely on crypsis or refuge seeking (Everett and Ruiz 1993, Pitcher 1993). Schooling relies on the rapid transfer of information between individuals, a process that breaks down in structurally complex environments. Populations originating from habitats with varying levels of habitat complexity should show correspondingly variable preferences for such habitats in the laboratory. Rainbowfish derived from a large open lake (Lake Tinaroo) avoided areas containing artificial submerged aquatic vegetation in laboratory experiments, whereas rainbowfish derived from a structurally complex environment (Dirran Creek) showed strong preferences for artificial vegetation (Brown and Warburton 1997). It remains unknown how the differences in habitat preferences come about, but most other anti-predator responses form by a combination of socially or genetically inherited traits that are fine-tuned through learning.

It is difficult to separate the roles of genes and experience in the development of behavioural patterns (Huntingford 1986), however there is some evidence that suggests that some components of anti-predator behaviours are inherited. For example, Seghers (1974) raised guppies (*Poecilia reticulata*) in the laboratory in a controlled, predator free environment yet the schooling tendencies of the fish remained the same three generations later. Similarly, the overhead flight responses of sticklebacks (*Gasterosteus aculeatus*) requires no experience to develop (Giles 1984). On the other hand, Dill (1974) suggested that the flight responses of the zebra danio (*Brachydanio rerio*) are modified with experience. Furthermore, anti-predator behaviour in minnows (*Phoxinus phoxinus*) has shown to be partially inherited but alter significantly through learning (Magarran 1990).

In this Chapter I set out to determine to what extent (if at all) habitat choice is controlled by individual experience. Are habitat preferences inherited or can varying the environment in which the animals are raised influence them? In other words, are habitat preferences plastic or fixed? Furthermore, I wished to determine if habitat choice was influenced by the presence of predators. Are animals that have grown up in a complex environment more likely to use complex structure to avoid predators?

3.3 METHODS

3.3.1 Experiment 1: Cross generation comparisons in the use of cover

Rainbowfish were bred from two populations, Dirran Creek and captive stock, collected for an earlier study (Brown and Warburton 1997). All fish were fed on tropical flake food and vinegar worms (*Turbatrix aceti*) until they reached an average standard length of around 2cm. The grow-out tanks were completely bare with the exception of a gravel substrate and a bubble filter in one corner. The light regime was approximately 10:14 D:L. Once the fish had reached an size suitable for observation under the video camera (around 2cm standard length), a group of five fish were taken at random from either of the two populations and placed in the experimental tank for 15 min. Four groups of fish were tested for each population. The dimensions of the experimental tank were 30cm x 60 cm. The depth was maintained at 20 cm and water conditions were identical to the enclosure tanks. The test tank contained two small areas of green wool suspended from the surface by polystyrene floats. The bottom of the tank was marked with a five by five cm grid and a camera was mounted above. A smaller tank abutted the test tank at one end. A trap door could be raised and lowered remotely to expose the rainbowfish to a large goldfish (standard length = 12cm) which was located in the smaller tank. The dimensions of the smaller tank ensured the goldfish was always close to the test arena while still enabling it to move about freely. The goldfish was chosen for this study because it is highly conspicuous being bright orange and highly mobile. Previous pilot studies suggest that juvenile rainbowfish find large goldfish very threatening and on several occasions the goldfish ate a juvenile rainbowfish (hence the partition between the goldfish and rainbowfish). The entire set up was surrounded and covered by a cream coloured sheet. The sheet eliminated any variations in light levels within the tank and prevented glare reflecting off the water into the camera. The sheet also served to minimize distractions from outside. After the 15 min settling period, the camera was turned on and recording commenced. After a further 10 min, the trap door was opened to expose the rainbowfish to the potential predator (the goldfish). The exposure lasted 10 min. Every min for the ten min control and exposure periods the following data were taken; EGS (Elective Group Size), SDI (Shoal Dispersal Index), distance to predator, and the mean number of fish utilising cover. The results were analysed using Wilcoxon-Mann-Whitney tests. Data for the control and predator present periods were combined, tested for normality and analysed using a one-way ANOVA. The results compared to the parental scores (Brown and Warburton 1997).

3.3.2 Experiment 2: Manipulation of the rearing environment

Rainbowfish were collected from three locations (North Johnstone River, upper and lower South Johnstone River) and bred in captivity. The North Johnstone and lower South Johnstone both contained *M. splendida* populations while the upper South Johnstone contained *M. eachamensis* (see maps in Chapter 4, Figure 4.A).

The populations represented in this experiment live in areas subject to different levels of predation pressure. The lower South Johnstone River contained a large number of predators including jungle perch, sooty grunter and mouth almighty (predator density = 0.36 m^{-3}). The North Johnstone River also contained predators, but only about a third as many as the lower South Johnstone River (predator density = 0.12^{-3}). The North Johnstone River site did not harbour jungle perch. The Upper South Johnstone was predator free (predator density data provided by B. Pusey pers. comm.). The habitat characteristics of all three sites were similar. Cobbles, fine gravel and areas of silt in both the South Johnstone sites dominated the river bed, while the North Johnstone also had a large amount of bedrock. In terms of habitat complexity, the North Johnstone is the most complex (index = ten), while the upper and lower South Johnstone complexity scores were eight and seven respectively. The habitat complexity index was calculated from the site proforma. There are 11 potential types of instream cover and each may receive a score from four (very abundant) to zero (absent). The total score for all 11 cover types is then summed to provide the habitat complexity index. Only the upper South Johnstone site had small beds of submerged macrophytes. Paragrass had grown into the water at all three sites and rainbowfish are commonly associated with this type structure.

The fry produced by the captive-breeding program were divided into groups of 20 individuals and transferred from the breeding tank to smaller enclosures immediately after hatching. The enclosures were fish tanks measuring 22 cm x 47 cm, with a water depth of 20 cm. Each enclosure was divided in half by an opaque partition. On one side of the partition a green woolen mop was provided to simulate complex structure while the other side remained bare. The water temperature was 23° C and pH was close to neutral. The floors of the tanks were covered in aquarium stones and aeration was provided by air-stones. Half of the fry from each population were placed into one side containing structure while the other half where placed into the bare side. All fish were fed on tropical flake food and vinegar worms (*Turbatrix aceti*). The light regime was approximately 10:14 D:L. Six groups of fish from both the upper and lower South Johnstone rivers and 10 groups from North Johnstone river were used in the experiment. Of these only four, two and seven groups remained after 6 months, and had varying numbers of individuals (Table 3.1). Fish were moved between like groups to supplement losses due to natural mortality. Interestingly, significantly more fry survived when structure was present in the tanks (Wilcoxon-Mann-Whitney test; Wx = 25, m = 6, n = 7, $P = 0.014$).

After six months the fry had reached a length of 2-3 cm. There were marked differences in growth rates within groups but there was no substantial difference in mean length between populations. A group of fish (see Table 3.1) was selected at random and placed into the test tank (described above). The test tank contained two small areas of green wool suspended from the surface by polystyrene floats. One of these floats was always derived from the test group's home tank. After a 30 min acclimation period the camera was turned on and the location of every individual fish was recorded every 30 sec for 15 min.

After the "control" period a trap door was raised to expose the goldfish (a potential predator). Recordings continued for a further 15 min. Control periods were always conducted first, as the behaviour of animals changes after exposure to a predator (see Chapter 4).

The video recordings were reviewed at a later time. The mean distance of the shoal to the barrier separating the two tanks was calculated for control and exposure periods as was the mean proportion of time the fish spent in the woolen mops.

Kruskal-Wallis tests were conducted on the data to investigate the effect of shoal size on the distance to the barrier and the proportion of time spent in the wool. Differences between populations, control and predator periods, and treatments (fish grown up in Wool versus No Wool environments) were examined using Wilcoxon-Mann-Whitney tests.

Table 3.1. The number of fish from each population remaining after the 6 months growing period, their tank environment allocation, species and the group number. M.spl. = *M. splendia* and M. each. = *M. eachamensis*.

Group #	Population	Species	Structure	No. of fish
1	L. Sth. Johnst.	M. spl.	Wool	5
2	L. Sth. Johnst.	M. spl.	Wool	5
3	L. Sth. Johnst.	M. spl.	Wool	6
4	L. Sth. Johnst.	M. spl.	No	4
1	Nth. Johnstone	M. spl.	No	1
2	Nth. Johnstone	M. spl.	No	3
3	Nth. Johnstone	M. spl.	No	4
4	Nth. Johnstone	M. spl.	No	4
5	Nth. Johnstone	M. spl.	Wool	3
6	Nth. Johnstone	M. spl.	Wool	5
7	Nth. Johnstone	M. spl.	Wool	5
1	Up. Sth. Johnst.	M. each.	Wool	5
2	Up. Sth. Johnst.	M. each.	No	1

3.4 RESULTS

3.4.1 Experiment 1: Cross generation comparisons in the use of cover

The two populations showed similar trends in their response towards the predator in all variables recorded. Fish from both populations increased their EGS, the distance to the predator, and the time spent in cover once exposed to the predator. SDI decreased for both populations. However, only the distance to the predator was statistically significant (H{1, N=8} = 5.333, *P* = 0.021 and H{1, N=8} = 3.000, *P* = 0.043 for captive fish and Dirran Creek fish respectively). This is not surprising considering the small sample size (n = 4 for each population). At no stage was there a difference between the responses of the two populations. One striking result however is the dramatic differences between the two populations in their use of cover. If the two periods (control and predator) are combined to increase the power of the statistical analysis we find that the Dirran Creek fish spent significantly more time in cover than the captive fish (df = 1, F = 6.761, *P* = 0.021). Dirran Creek fish spent over 40% of their time in cover (well over that expected by chance 20%) compared to under 10% by the captive fish (Figure 3.1).

Brown and Warburton (1997) found that the wild caught Dirran Creek fish spent significantly more time in the quadrants containing weed than the Captive adult stock (Figure 3.2). In their 1997 experiments the Dirran Creek fish spent significantly more time in the cover than was expected by chance (36%).

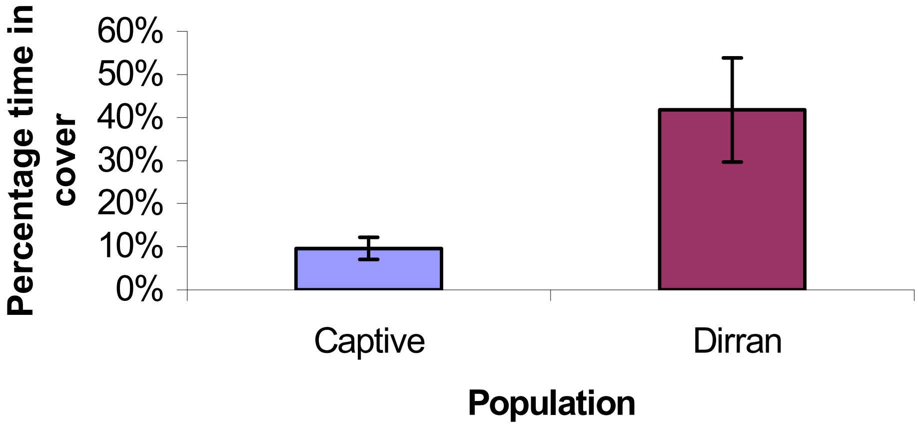

Figure 3.1. The mean (± se) amount of time fish from each of the two populations spent in cover. Data is combined for the predator and control periods. Fish are expected to spend 20% of their time in cover if they move randomly.

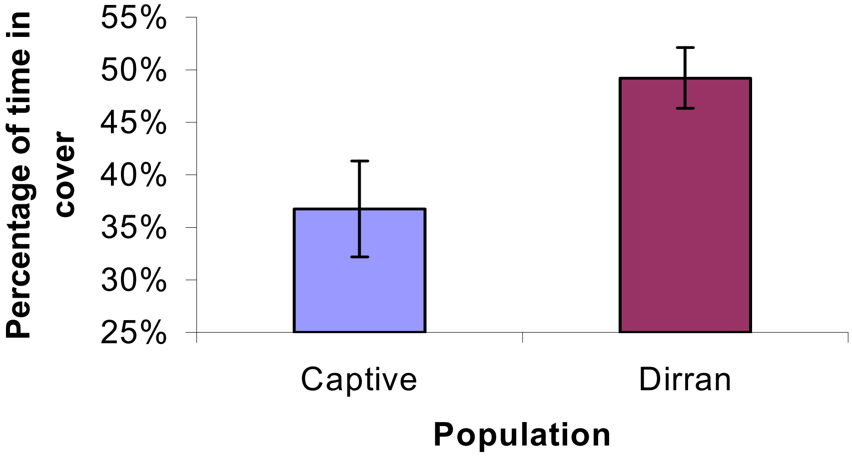

Figure 3.2. The mean (± se) percentage of time spent in cover by adult Captive and Dirran Creek rainbowfish (from Brown and Warburton 1997). Fish are expected to spend 36% of their time in cover if they move randomly.

3.4.2 Experiment 2: Manipulation of the rearing environment

In order to examine the effect of having grown up with and without the wool in the tank all the populations were lumped together to increase the power of the test. There was no significant difference in the amount of time fish spent in the wool or in the distance from the predator divide (Table 3.2, Figures 3.3 and 3.4). This is almost certainly due to the large variation caused by the differences between populations. Only the North Johnstone River data were extensive enough to be considered independently. These data showed no significant difference between the two treatments (wool versus no wool) (Table 3.2), but there was a significant preference for the wool during the predator period shown by those fish which had grown up without wool (Figure 3.5).

	Period	**Data**	**Wx**	**n**	**m**	***P*- value**
Time in cover	Control	All pop's	36	6	7	0.432
	Predator	All pop's	53	6	7	0.133
Distance	Control	All pop's	39	6	7	0.721
	Predator	All pop's	49	6	7	0.353
Time in cover	Control	Nth. Johnstone	14	3	4	0.800
	Predator	Nth. Johnstone	10	3	4	0.596
Distance	Control	Nth. Johnstone	17	3	4	0.112
	Predator	Nth. Johnstone	9	3	4	0.337

Table 3.2. The effect of treatment (i.e. growing up with or with out wool in the tank) on the time spent in cover (wool) and the distance to the predator for the control and predator exposure periods. Wx is the Wilcoxon-score, n is the sum of the major ranks and m is the sum of the minor ranks.

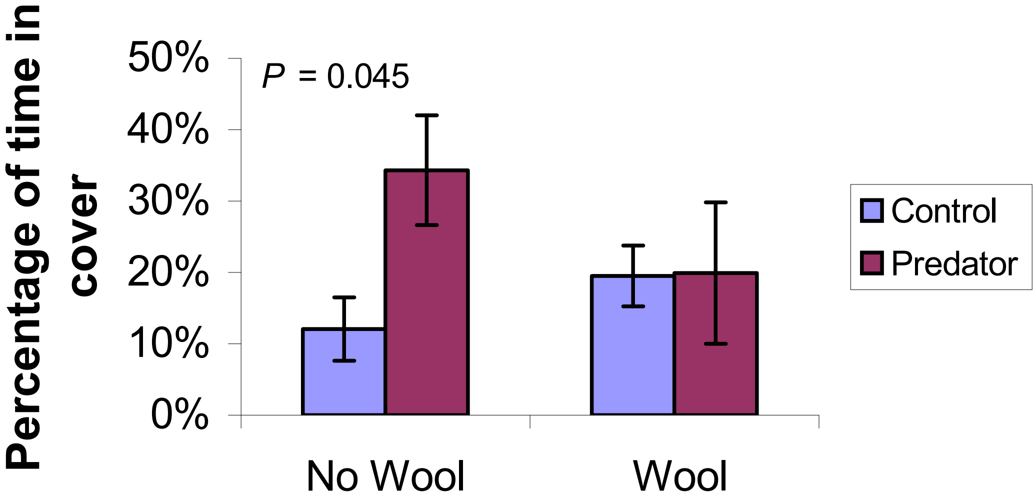

Figure 3.3. The amount of time all fish (combined populations) spent in cover during the control period and when the potential predator was present. Two treatments are represented. No Wool fish grew up in enclosures completely devoid of cover, while the Wool treatment fish grew up with green woolen mops present.

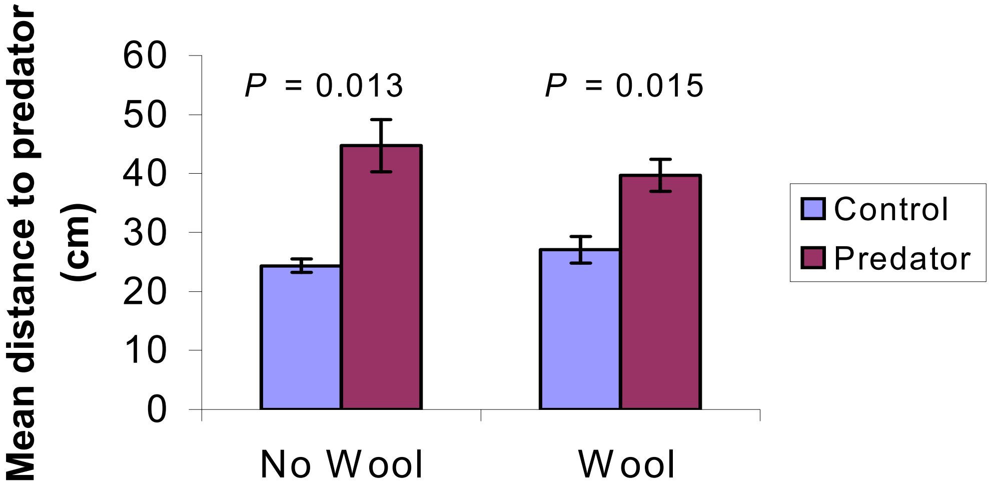

Figure 3.4. The average distance to the divide separating the predator compartment from the rainbowfish compartment (±se). The data were combined from all populations.

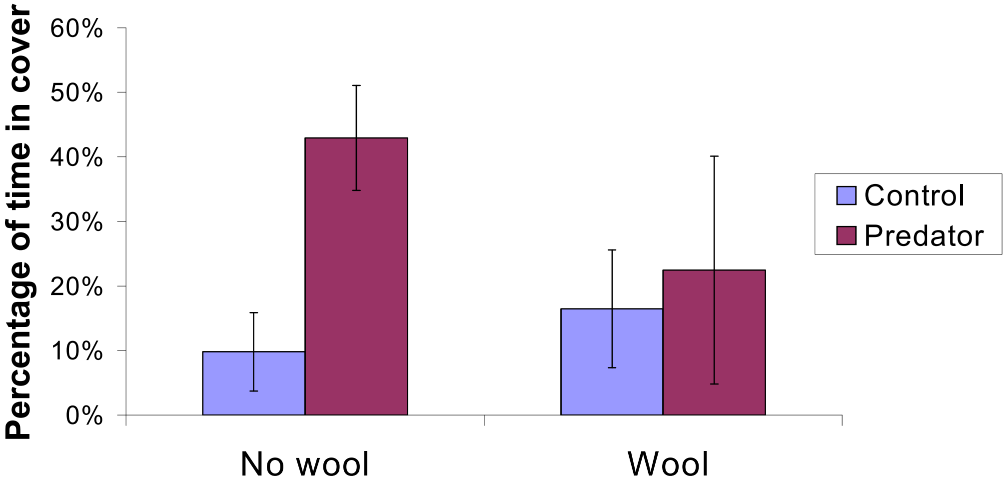

Figure 3.5. The proportion of time the fish from the North Johnstone River spent in the wool (cover) during the control period and during exposure to the potential predator.

Fish that had grown up with no wool in their tanks spent significantly more time in cover when the goldfish was present compared to the control period (Table 3.3). Fish that had grown up with wool in their tanks showed an elevated amount of time spent in wool compared to the other No Wool fish during the control period, but did not significantly alter the amount of time spent in cover when exposed to the predator (Figure 3.3, Table 3.3). In terms of the distance to the predator, both Wool and No Wool fish were significantly further from the partition when the goldfish was exposed to them compared with the control period (Figure 3.4, Table 3.3). At the population level, none of the populations altered the amount of time spent in cover significantly when exposed to the predator (Table 3.3). The lower South Johnstone fish showed no change in their use of cover as a result of exposure to the goldfish, spending about 15 % of their time in cover for both periods. In contrast, the North Johnstone fish spent the same amount of time in cover as the lower South Johnstone fish during the control period, but increased the amount of time spent in cover to 35% when exposed to the predator. The upper South Johnstone decreased the amount of time spent in cover from 22.5% to 7.5% when exposed to the predator (Figure 3.6, Table 3.3). However, none of these exposure related differences in cover use were statistically significant (Table 3.3).

Fish from both populations of *M. splendida* (Nth and Lower Sth. Johnstone) moved significantly further from the divide when exposed to the predator compared with the control period, whereas the M. *eachamensis* (Up. Sth. Johnstone) fish did not (Figure 3.7, Table 3.3). The North Johnstone showed a significant increase in the distance to the predator divide when the goldfish was revealed. There was also a trend to increase the amount of time spent in cover (Table 3.3, Figure 3.5).

There was no significant effect of shoal size on either the amount of time spent in cover or the distance to the divide during the control and predator periods, or in the Wool and No Wool treatments (Table 3.4). These data were combined for all populations.

	Treatment	**Data**	**Wx**	**n**	**m**	***P*- value**
Time in cover	No wool	All pop's	26	6	6	0.045
	Wool	All pop's	63	7	7	0.201
Distance	No wool	All pop's	23	6	6	0.013
	Wool	All pop's	33	7	7	0.015
Time in cover	Combined	L. Sth. Johnst.	18	4	4	1.000
Distance	Combined	L. Sth. Johnst.	10	4	4	0.030
Time in cover	Combined	Nth. Johnstone	40	7	7	0.125
Time in cover	No Wool	Nth. Johnstone	11	4	4	0.057
Distance	Combined	Nth. Johnstone	30	7	7	0.005
Distance	No Wool	Nth. Johnstone	10	4	4	0.029
Time in cover	Combined	Up. Sth. Johnst.	7	2	2	0.245
Distance	Combined	Up. Sth. Johnst.	3	2	2	0.245

Table 3.3. The effect of exposure to the potential predator (i.e. predator versus control periods) on the time spent in cover (wool) and the distance to the predator. The first four rows of the results are based on lumped data for all three populations and have been divided into the two treatments (wool versus no wool) for analysis. The final eight rows contain results for each population. The two treatments were lumped for both of the South Johnstone sites. The data for the No Wool treatment was also considered separately for the Nth Johnstone fish.

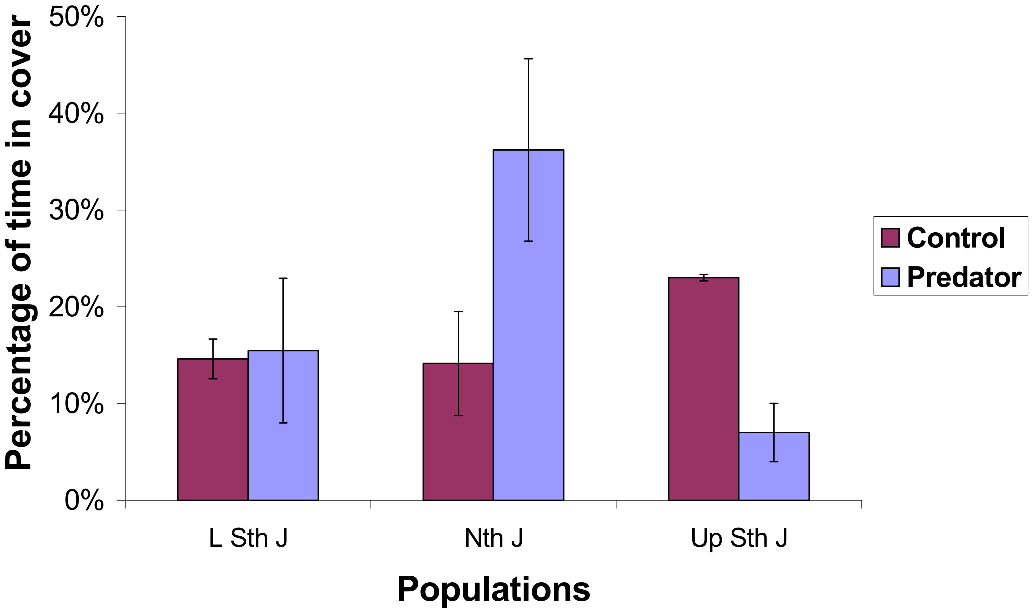

Figure 3.6. The mean (± se) amount of time fish from all three populations, Lower South Johnstone (L Sth J), North Johnstone (Nth J) and Upper South Johnstone (Up Sth J), spent in the cover during the control and predator periods.

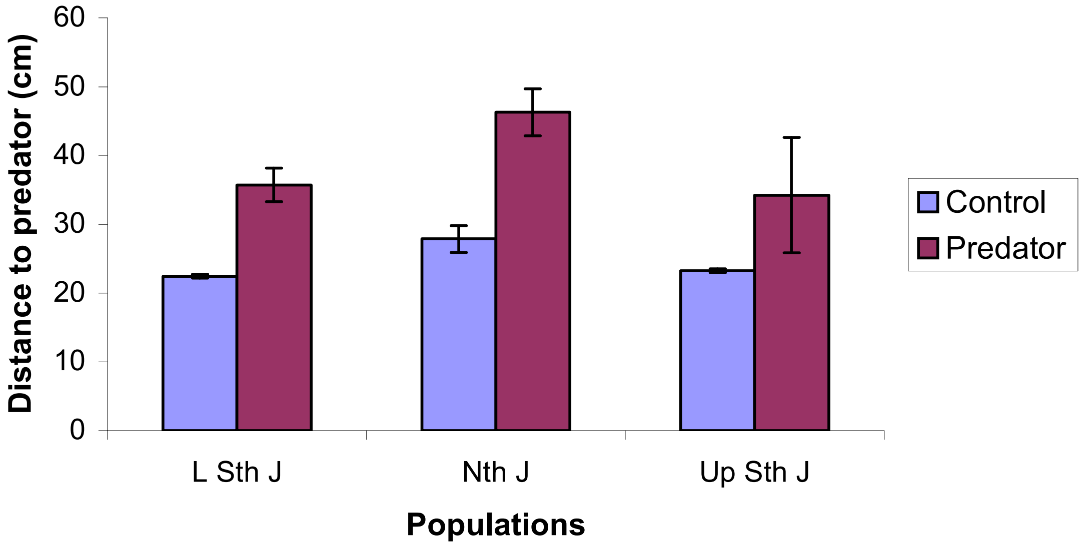

Figure 3.7. The mean (± S.E.) distance to the predator divide shown for all three populations, Lower South Johnstone (L Sth J), North Johnstone (Nth J) and Upper South Johnstone (Up Sth J), during the control and predator present periods.

Table 3.4. Results of a Kruskal-Wallis analysis of the effect of shoal size on the mean amount of time the fish spent in cover and the mean shoal distance to the divide. Data are combined for all populations.

Variable	Period	Treatment	H - statistic	*P*-value
Time in Cover	Control	Wool	H(2, N = 6) = 0.429	0.807
		No wool	H(2, N = 7) = 2.829	0.243
	Predator	Wool	H(2, N = 6) = 2.381	0.304
		No wool	H(2, N = 7) = 2.356	0.308
Distance to divide	Control	Wool	H(2, N = 6) = 2.143	0.343
		No wool	H(2, N = 7) = 0.257	0.879
	Predator	Wool	H(2, N = 6) = 1.238	0.539
		No wool	H(2, N = 7) = 0.257	0.879

3.5 DISCUSSION

The results of the first experiment indicate clearly that a preference for the use of cover is maintained in the offspring of the adult fish. Brown and Warburton (1997) found that Dirran Creek fish showed a significant preference for cover and they utilised cover significantly more than fish from the captive stock. Furthermore, the captive stock utilised cover as would be expected if they moved randomly throughout the experimental arena. The juveniles tested in the first experiment repeated this pattern, although the captive juveniles perhaps avoided cover a little more strongly than their parents did. This provides strong evidence that the use of cover as part of the anti-predator response is inherited, even when the juveniles are denied access to cover as they grow up.

Fish from both populations showed significant changes in their behaviour when exposed the predator. Both groups of fish increased their elective group sizes and were further from the predator partition when the predator was exposed to them. This unexpected response was also found in the adult fish of both these populations even though neither has been subject to predation pressure in the wild for perhaps a million years or so.

The results of the second experiment indicate that there was no significant effect of individual experience on the use of cover, but there was an interesting treatment-period interaction. Fish that had grown up with or without the woolen mops in their enclosures behaved relatively similarly, particularly in terms of the distance they maintained from the predator partition. Fish from both treatments significantly increased their distance to the predator after it was exposed to them, as did the fish in Experiment 1. However, fish that had grown up with the wool utilised the cover more during the control period than those that had not been exposed to the wool before. Surprisingly, the No Wool fish significantly increased the use of the cover when the predator was present, whereas the Wool fish maintained a similar level of occupancy. This result adds further weight to the notion that the use of complex habitat is probably predominantly genetically inherited but may be partially altered by individual experience.

Large variability in the results was evident during the predator periods for both the Wool and No Wool treatments. This stemmed primarily from large population differences in responses and also the variation within populations (i.e. between groups).

Fish from all populations responded to the presence of the predator by increasing the distance between themselves and the threat. The difference between control and predator periods was significant for both the *M. s. splendida* populations. Both these populations suffer from considerable predation pressure in the wild. The upper South Johnstone result may have been insignificant due to low sample sizes, however, the relatively high use of cover by these fish suggests that they were doing something a little different. Unlike the North Johnstone fish, the upper South Johnstone fish actually emerged from the cover rather than fleeing into it when they were exposed to the predator. This result is very similar to other results obtained when other predator-naïve rainbowfish populations have been investigated (see Chapter 2 and Brown and Warburton 1999).

Of the two *splendida* populations, only the North Johnstone fish showed any change in the use of cover between the control and predator periods. In the presence of a potential predator, fish from this population increased the amount of time spent in cover although not significantly so. The variation in the results stemmed from the differences in the two treatments. Only the fish from the No Wool treatment increased the amount of time spent in cover when exposed to the predator. In the wild the North Johnstone fish were commonly associated with structurally complex microhabitats, particularly paragrass that grows out from the bank and over the water. Why then was a similar result not seen in the wool treatment?

I think the answer to this question may lie in the set up of the experimental tank. It could be that the No-Wool fish avoided the wool during the control period since it was foreign to them, but, once exposed to the predator, their minor aversion of the wool was replaced by a more substantial avoidance response directed towards the predator. By moving further from the predator divide they were more likely to utilise the cover simply because the cover was placed at the back of the tank and extended some 20 cm down one side towards the predator. Thus the front two thirds of the tank were completely devoid of structure. There was a clear relationship between the distance from the predator and the amount of time spent in cover. In nearly all cases the mean distance to the predator divide during the predator period was over 40 cm, indicating that the fish were primarily utilising the area of tank which contained at least some structure. The use of cover increased correspondingly. Nevertheless this did not detract from the minor differences observed between the fish which had grown up under different conditions. Fish that had grown up with wool in their tank utilised the structure at the same rate irrespective of the experimental period.

The differences in anti-predator responses displayed by members of the five populations suggest that a proportion of their anti-predator response was inherited. The adults from both the *splendida* locations showed strong anti-predator responses and tended to prefer to school in relatively open areas (Chapter 4). All the fish used in this experiment grew up in identical conditions, yet substantial inter-population differences still existed. Others have shown that the anti-predator responses of small fish are partially inherited (Seghers 1974, Giles 1984, Magurran 1990, Magurran and Seghers 1990), but may be substantially influenced by individual experience (i.e. learning) (Dill 1974, Csanyi 1985, Tully and Huntingford 1987). European minnows reared in the lab but descended from predator sympatric fish not only show relatively strong initial responses to a predator model, but also showed the greatest ability to modify their anti-predator behaviour (Magurran 1990). Altering the anti-predator response may also occur through complacency rather than through learning. For example, significant population differences occur between adult guppies from the Upper and Lower Aripo River, but there is no difference between these fish at birth (Magurran and Seghers 1990). The discrepancy may be explained by a lack of reinforcement as the Upper Aripo fish grow in a predator-free environment.

Unfortunately, a large number of fry died during the grow-out period, which substantially reduced the power of the second experiment. The majority of the deaths probably occurred due to overcrowding although it is clear that more fry survived when the woolen mops were placed in the tanks. The wool probably provides a substrate upon which microorganisms can grow, allowing the fish to supplement their diets by grazing. Even considering the number of deaths, the current experiments strongly suggest that the use of cover is primarily genetically predetermined and only slightly influenced by personal experience, but further work using controlled breeding experiments is clearly required to confirm this.

References

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